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THE RELATIONSHIP BETWEEN MIR-196A2 POLYMORPHISM AND COLORECTAL CANCER RISK

MIR-196A2 POLİMORFİZMİ İLE KOLOREKTAL KANSER RİSKİ ARASINDAKİ İLİŞKİ

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ABSTRACT

Objective: MicroRNAs are small endogenous, non-coding, single-stranded posttranscriptional RNA molecules. The discovery of microRNAs has made new contributions to cancer diagnosis and treatment. These microRNAs reported as a responsible for colorectal cancer development with several epigenetic changes. In this study, it was aimed to evaluate the relationship between the polymorphism of miR-196a-2 polymorphism rs11614913 and colorectal cancer in Turkish population.

Methods: Two hundred colorectal cancer patient (124 colon cancer and 76 rectal cancer) and 240 health control individuals were included in our study, which was planned as a hospital based retrospective cohort study. MiR-196a2 polymorphism in peripheral blood samples has been determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method. Significance of the results has been evaluated by using SPSS (20.0 SPSS Inc., Chicago, IL, USA.) statistical program.

Results: miR-196a2 C / C + C / T genotypes was found to be associated with the risk of colorectal cancer development (p: 0.001; OR: 2.04, 95% CI: 1.293-3.236). The subgroup analysis, showed that the C / C + C / T genotype increased the risk of colon cancer development 2.11 times (p: 0.016; 95% CI: 1.136-3.918) and rectal cancer 2.86 times (p: 0.011; 95% CI:1.242-6.592). The relationship between any clinicopathological features of colorectal cancer and the frequency of the C / C + C / T genotype of miR196a2 was not statistically significant (p> 0.05).

Conclusion: This study supports that miR-196a2's C / C + C / T genotypes is related with increased colorectal cancer development risk.

Keywords: Colorectal cancer, MicroRNA, miRNA-196a2 polymorphism

ÖZ

Amaç: MicroRNA'lar küçük, endojen, kodlanmayan, tek sarmallı posttranskripsiyonel RNA molekülleridir. MikroRNA'ların keşfi, kanser teşhis ve tedavisine yeni katkılar sağlamıştır. Bu miRNA'ların çeşitli epigenetik değişiklerle kolorektal kanser gelişiminden sorumlu olduğu saptanmıştır. Bu çalışmada Türk toplumunda miR-196a2'nin rs11614913 polimorfizmi ile kolorektal kanser arasındaki ilişkinin değerlendirilmesi amaçlanmıştır.

Yöntem: Hastane bazlı retrospektif kohort çalşması olarak planlanan çalışmamıza 200 kolorektal kanser hastası (124 kolon kanseri ve 76 rektum kanseri) ve 240 sağlıklı kontrol bireyler dahil edilmiştir. Periferik kan örneklerinde miR-196a2 polimorfizmi, polimeraz zincir reaksiyonu ve restriksiyon fragman uzunluk polimorfizmi yöntemi ile belirlenmiştir. Sonuçların anlamlılığı SPSS (20.0 SPSS Inc., Chicago, IL, USA.) istatistik programı kullanılarak değerlendirilmiştir.

Bulgular: MiR-196a2 C/C + C/T genotiplerinin kolorektal kanser gelişim riski ile ilişkili olduğu saptanmıştır (p: 0.001; OR: 2.04, %95 CI: 1.293-3.236). Altgrup analizleri ise ise C/C+C/T genotipinin kolon kanseri gelişim riskini 2.11. kat (p: 0.016; %95 CI: 1.136-3.918) ve rektum kanserini ise 2.86 kat (p: 0.011; %95 CI: 1.242-6.592) arttırdığını göstermiştir. Kolorektal kanserin herhangi bir klinikopatolojik özelliği ile miR-196a2'nin C/C+ C/T genotipinin sıklığı arasında istatistiksel olarak anlamlı ilişki saptanmamıştır (p>0.05).

Sonuç: Bu çalışma miR-196a2'nin C / C + C / T genotiplerinin artmış kolorektal kanser gelişim riski ile ilişkili olduğunu desteklemektedir.

Anahtar Kelimeler: Colorectal cancer, MicroRNA, miRNA-196a2 polymorphism

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Introduction

Colorectal cancer (CRC) is one of the most frequently diagnosed cancer which is third most common cancer in men and second most common cancer in women.[1] Although its incidence and mortality rates have increased in recent years, its mobidity and mortality risk has decreased in countries with screening programs.[2]

The etiology of CRC is multifactorial including genetic, epigenetic, lifestyle factors and environmental factors.[3] As one of epigenetic factors, single nucleotide polymorphisms (SNPs) in miRNA's have been reported.[1] MiRNA's are small, non-coding group of RNAs which functions both in tumor supressions and oncogenic activity.[4] These miRNA's act on mRNA's and MiRNA's can play an important role in tumor initiation, proliferation, apoptosis, migration, metastasis, response to chemotherapy and radiotherapy in several cancers.[3,5] SNPs in miR-196a2 in CRC, has been studied in different countries and population groups for the risk of development of CRC. While some studies have shown susceptibility to CRC, some studies have not found any susceptibility.[2,4]

In this study, we aimed to evaluate the relationship between the polymorphism of miR-196a2 and CRC in Turkish population.

Methods

Patients

Two hundred CRC patient (124 colon cancer and 76 rectal cancer), who were diagnosed by endoscopic evaluation, histopathological examination, imaging techniques, and 240 healthy control individuals were included in our study who admitted to Istanbul University, Istanbul Medical Faculty, General Surgery Department which was planned as a hospital based retrospective cohort study. The study protocol was approved by the Biruni University Ethical Committee. This study was funded by Istanbul University Scientific Investigation Projects No: 5821

Genotyping

Blood samples from all CRC patients and healthy control groups were stored in Ethylenediaminetetraacetic acid disodium salt (EDTA) tubes. Genomic DNA was extracted from peripheral whole blood according to salting-out technique. MiR-196a2 polymorphism in peripheral blood samples has been determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method.

Genotyping was performed by the procedures of polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) using MspI restriction enzyme. PCR was performed with using primers (miR 196a-2 ''F'' 5' CCC CTT CCC TTC TCC TCC AGA TA 3' and ''R'' 5' CGA AAA CCG ACT GAT GTA ACT CCG 3'). The PCR product was then digested using MspI restriction enzyme (Thermo Fisher Scientific, Inc., Pittsburgh, PA, USA) for 16 h at 37˚C. The resulting fragments were separated by electrophoresis on a 3% agarose gel. The CC genotype produced two fragments (125 and 24 bp), the TT homozygote produced one 149 bp fragment and the TC heterozygote produced three fragments (125, 149, and 24 bp).[6]

Statistical analysis

Significance of the results has been evaluated by using SPSS (20.0 SPSS Inc., Chicago, IL, USA.) statistical program.

Statistical analyses were performed using the R (R Core Team, 2017, Vienna, Austria) program. p-Values less than 0.05 were considered to be statistically significant. Statistical analyses were performed using the SPSS software package (revision 20.0 SPSS Inc.,Chicago, IL, USA.). Data are expressed as mean+SD. Differences in clinicopathological characteristics between patients and controls were tested by chi-square test for categorical data and Student's t-test for numerical data. Odds ratio (OR) and 95% confidence interval (CI) for the association between genotype and CRC was computed. A two-sided p-value of less than 0.05 was considered statistically significant.

Results

Characteristics of colorectal cancer patients and control groups

Two hundred CRC patients and 240 controls were studied. Characteristics of CRC patients and control groups were given in Table 1. There was no significant difference in the characteristics between CRC patients and control groups (p>0.05) (Table 1).

MiR-196a2 polymorphism

miR-196a2 C / C + C / T genotypes was found to be associated with the risk of colorectal cancer development (p: 0.001; OR: 2.04, 95% CI: 1.293-3.236). The subgroup analysis, showed that the C / C + C / T genotype increased the risk of colon cancer development 2.11 times (p: 0.016; 95% CI: 1.136-3.918) and rectal cancer 2.86 times (p: 0.011; 95% CI:1.242-6.592). The relationship between any clinicopathological features of colorectal cancer and the frequency of the C / C + C / T genotype of miR196-a2 was not statistically significant (p> 0.05). (Table 2)

Table 1. Characteristics of colorectal cancer patients and control groups

		Colorectal cancer patients (n=200)		Control group(n=240)	p value
		Colon cancer (n=124)	Rectal cancer (n=76)		
Sex	**Female**	49 (39.5%)	28 (36.8%)	90 (37.5%)	
	Male	75 (60.5%)	48 (63.2%)	150 (62.5%)	0.457
Smoking	**Yes**	40 (32.2%)	27 (35.5%)	89 (37.1%)	
	No	84 (67.7%)	49 (64.5%)	151 (62.9%)	0.910
Alcohol consumption	**Yes**	21 (16.9%)	17 (22.4%)	36 (15%)	
	No	103 (83.1%)	59 (77.6%)	204 (85%)	0.680
T Staging	**T 1**	6 (4.8%)	3 (3.9%)		
	T 2	24 (19.4%)	10 (13.2%)		
	T 3	60 (48.4%)	37 (48.7%)		
	T 4	34 (27.4%)	26 (34.2%)		
Lymph node involvement	**N 0**	48 (38.7%)	14 (18.4%)		
	N 1	32 (25.85%)	18 (23.7%)		
	N 2	44 (35.5%)	44 (57.9%)		
Distant metastasis	**Yes**	13 (17.1%)	17 (13.7%)		
	Absent	63 (82.9%)	107 (86.3%)		
Tumor differentiation	**Well or moderately**	84 (67.7%)	52 (68.4%)		
	Poorly	40 (32.3%)	24 (31.6%)		

*Values are given as percentage (%) in the table. p-value less than 0.05 was considered as significant. Nodal involvement p:0.08

Table 2. The relationship between genotype/alleles and the colorectal cancer risk

Genotype /alleles	Colorectal cancer patients (n=200)		Control group (n=240)	p value
	Colon cancer (n=124)	Rectal cancer (n=76)		
CC	61	37	95	**0.012***
CT	46	34	91	0.240
TT	17	5	54	0.280
CC + C / T	107	71	186	**0.003***
TT + C / T	73	39	14	0.140

*P-value less than 0.05 was considered as significant. Bold values indicate statistical significance.

Discussion

CRC is one of the most common cancer types and its management is still challenging. Screening programs helps to decrease incidence. Although colonoscopy is generally used for screening it is invasive and expensive.[7] Epigenetic factors such as SNPs in miRNA's that may be useful for diagnosis, prognosis and follow-up. Different types of miRNA's can have spesific correlation with different types of tumor.

miR-196a2 has garnered particular attention due to its potential involvement in oncogenesis, tumor progression, and metastasis in CRC. A growing body of evidence suggests that miR-196a2 modulates the Wnt/β-catenin signaling pathway, a key driver of CRC development. Aberrant activation of this pathway has been shown to contribute to CRC by promoting cellular proliferation and inhibiting apoptosis.[8]

Our study supports that miR-196a2's C / C + C / T genotypes is related with increased risk of CRC development in Turkish population. Indeed, two different meta-analysis reported that miR-196a2 might play a role in pathogenesis of CRC in Iranian and Asian population in concordence of our findings in Turkish population.[9,10] Beside this, miR-196a2 polymorphism was found to be not related with the increasing of CRC development ontrary in Chinese population.[4]

The potential links between several miRNA gene polymorphisms, including miR-27a, miR-146a, miR-196a2, miR-492, and miR-608 and CRC development were investigated in a recent study.[11] Despite prior evidence suggesting that these miRNAs play crucial roles in cancer biology by regulating key oncogenic pathways, the study's findings indicate that genetic variations in these specific miRNAs do not substantially influence CRC susceptibility. These results highlight the complexity of miRNA involvement in cancer and suggest that while miRNAs may contribute to tumor biology, their polymorphisms alone may not be reliable markers for CRC risk.[11]

miRNA's are also promising factors for diagnosis and new treatment strategies.[5] From a clinical perspective, miR-196a2 holds promise as a biomarker for colorectal cancer. Its overexpression in tumor tissues, as well as its detectability in serum, makes it a candidate for non-invasive diagnostic tests.[12]

Also Ge et al., mentioned that high miR-196a2 expression is strongly associated with poor prognosis in colorectal cancer (CRC) patients, consolidating its role as a significant biomarker in predicting clinical outcomes. This supports the hypothesis that miR-196a2 not only contributes to tumor progression through key oncogenic pathways but also exacerbates aggressive tumor behavior, ultimately leading to worse clinical outcomes.[13] miRNAs represent promising therapeutic targets due to their ability to modulate gene networks, making them integral to novel anti-cancer strategies. [14]

MiRNAs are pivotal in modulating key processes such as cell proliferation, invasion, and metastasis, all of which are central to CRC progression. Specific miRNA signatures associated with CRC, such as the downregulation of tumor-suppressive miRNAs and upregulation of oncogenic miRNAs, contribute to the complex molecular mechanisms underlying tumor growth and metastatic potential. For instance, altered expression of miRNAs like miR-21, miR-200, and miR-34a has been linked to enhanced epithelial-mesenchymal transition (EMT), increased stemness and resistance to apoptosis in CRC. These findings suggest that targeting specific miRNA pathways could offer therapeutic benefits in controlling tumor progression and preventing metastasis. [15]

The role of exosomal miRNAs in cancer progression, particularly in chemoresistance and metastasis, has been increasingly recognized in recent years. Kulkarni et al. (2019) highlighted how exosomal miRNAs contribute to these processes by facilitating intercellular communication within the tumor microenvironment. These miRNAs can be transferred between cancer cells and surrounding stromal cells, promoting an environment conducive to tumor survival. In the context of chemoresistance, specific exosomal miRNAs can alter the expression of drug resistance genes, making cancer cells less susceptible to chemotherapy. For example, the upregulation of miRNAs like miR-21 and miR-221 has been associated with resistance to various chemotherapeutic agents. Additionally, exosomal miRNAs play a crucial role in enhancing metastatic potential by regulating genes involved in EMT and cell migration. This dual role of exosomal miRNAs in both drug resistance and metastasis underscores their significance as potential therapeutic targets, where inhibiting their activity could improve treatment outcomes by reducing resistance to chemotherapy and preventing cancer spread.[16]

Therefore, miRNAs can be used as a biomarker for early diagnosis, therapeutic target and also to determine prognosis due to the potential for metastasis.

There are some limitations of our study. First of all, our study size is small for the investigate the association of the risk of CRC development. Secondly, we have investigated only miRNA-196-a2 genotype of miRNA family.Thirdly, we have not investigate the other effects of miRNA-196-a2 genotype as metastasis, prognosis and progression of CRC.The effects of other MiRNA's family on metastasis, prognosis, occurrence of CRC as well as therapeutic option will be important to investigate fort he future studies.

In conclusion, our research highlight the pivotal role of miR-196a2 in the development and of CRC risk. Its involvement in key oncogenic pathways, its impact on chemoresistance, and its potential as both a prognostic biomarker and therapeutic target make miR-196a2 a focal point for future investigations in CRC. As the landscape of precision oncology evolves, targeting miR-196a2 could offer new hope for CRC patients, particularly those with poor prognostic factors. However, it is important to recognize that not all studies have reported a clear link between miRNA polymorphisms and CRC, as evidenced by research that found no significant association between various miRNAs and CRC risk. This discrepancy underscores the complexity of miRNA regulation in cancer and highlights the need for further, more comprehensive investigations. Future studies should focus on larger, more diverse populations and explore additional factors, such as environmental influences and gene-environment interactions, to better understand the precise role of miRNAs in colorectal cancer.

Compliance with Ethical Standards

The study was approved by Biruni University Ethical Committee (2024-BIAEK/03-30).

Conflict of Interest

The author declares no conflicts of interest.

Author Contribution

Conception and Design of Study: BCT, MTB, YB, EC. Data Acquisition: BCT, ÜZ, EC. Performing Laboratory Analysis: BCT, ÜZ. Data Analysis: BCT, EC. Drafting Manuscript: BCT. Critical Revision of Manuscript: EC. Final Approval: BCT. Supervision: ÜZ, MTB, YB, EC

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